DETERMINATION OF VITAMIN E IN BLOOD*

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(Received for publication, August 29, 1955)

A new colorimetric method for the estimation of vitamin E has been reported earlier (1). The method was found to be simpler than the ones already known, with the added advantages of having greater specificity and ease of operation. In the present investigation, this procedure has been adapted to the estimation of vitamin E in blood plasma or serum. The sensitivity has been considerably enhanced, permitting the estimation of 2 to 5 γ of vitamin E.

Materials and Methods

Reagents—
1. 5 per cent aqueous sodium hydroxide.
2. Petroleum ether (60–100°) purified according to the method of Mahon and Chapman (2).
3. Absolute alcohol purified by distillation from a flask containing KOH and KMnO₄ (20 gm. per liter of each).
4. Standard solution of pure dl-α-tocopherol (Hoffmann-La Roche) containing 5 γ per ml. in purified petroleum ether.
5. Color reagent, 0.394 gm. of phosphomolybdic acid per 100 ml. of glacial acetic acid.
6. Blood pipettes, 1 and 2 ml.
7. Beckman model DU quartz spectrophotometer with Corex absorption cells of 1 cm. light path.

Oxalated or whole blood is taken in Pyrex test-tubes 6 × 5/8 inches and centrifuged for 20 minutes at 2000 r.p.m. 1 or 2 ml. of serum or plasma and 0.5 ml. of 5 per cent aqueous sodium hydroxide are pipetted into a Pyrex test-tube and shaken well so that any esters of vitamin E, if

* A preliminary report was presented at the Forty-second session of the Indian Science Congress, January, 1955, at Baroda, India.
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1 The authors wish to thank Hoffmann-La Roche, Inc., Basel, Switzerland, and their agent Voltas, Ltd., India, for the generous gift of authentic samples of pure dl-α-tocopherol used in this work.
2 B. D. H. analar, mol. wt. 3940.8.
3 Merck's reagent grade, code No. 60.
present, may be saponified. The tubes are set aside for 6 hours, at the end of which time the solution is diluted with 10 ml. of distilled water, transferred to a 25 ml. separating funnel, and extracted twice with petroleum ether, 5 ml. each time. The petroleum ether extracts are combined and washed with distilled water until the washings are free of alkali as indicated by an absence of color with phenolphthalein.

The petroleum ether solution is transferred to a Pyrex test-tube connected to a vacuum line. Under partial vacuum the solvent is carefully removed to dryness.

To the residue in the test-tube is added 1 ml. of the phosphomolybdic acid reagent. Exactly 5 minutes after the addition of the phosphomolybdic acid reagent, the solution in the test-tube is diluted with 3 ml. of purified ethyl alcohol. The solution is well shaken and the color density is read in a Beckman model DU quartz spectrophotometer at 725 mp4 with Corex absorption cells and distilled water as a reference blank.

A standard containing 5 γ of tocopherol is treated simultaneously as stated above and the readings are taken against a reagent blank at the same wave-length.

Results

Twenty-five samples of normal human blood serum6 were analyzed for vitamin E content and the values obtained ranged between 0.361 and 0.412 mg. per 100 ml. Recovery experiments were conducted on rat blood serum. The amounts of vitamin E added ranged from 4 to 10 γ per ml. of serum. The recoveries averaged 98 per cent.

Discussion

In this paper a simplified procedure is described and the values obtained are in close agreement with the figures reported by previous methods (3).

The addition of alkali to the serum has been considered necessary, for the reason that the chromogenic reagent used in the present method is inactive with the esters of vitamin E present, if any.

The experimental evidence from studies conducted on the phosphomolybdic acid-vitamin E reaction indicates that it is one involving a specific complex formation at very low pH. The mechanism of the reaction has been worked out, the details of which will be published elsewhere. On

4 The absorption spectrum of the phosphomolybdic acid-vitamin E complex has been determined in the visible region, and the maximum was observed at 725 mp.

6 The standard is always prepared with free α-tocopherol, as the reaction with phosphomolybdic acid is absent with the esters of α-tocopherol.

6 We are indebted to Dr. M. D. Pathak and Dr. L. Marquis, of the B. Y. L. Nair Hospital and the National Medical College, Bombay, for the blood samples.
This basis it is felt that interference due to other reducing non-tocopherol components can be ruled out.

The present procedure was found to be free from interference owing to vitamin A, calciferol, cholesterol, and carotenoids (1). Under the conditions prescribed and with the given volumes and concentrations the method was found to be highly sensitive. Ease of operation and simplicity of color reagent aided in considerable saving in time required for individual assays.

We are greatly indebted to the Raptakos Medical Research Board for a research grant and a fellowship to one of us (P. P. N.)

SUMMARY

A new color reaction between phosphomolybdic acid and vitamin E has been used in developing a method for the estimation of blood vitamin E. The method is highly sensitive and specific, concentrations up to 2 γ per ml. being estimated with ease and simplicity.

BIBLIOGRAPHY

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